Supporting Online Material for

A yeast model establishes a functional connection between Aβ toxicity, endocytic trafficking and Alzheimer's Disease risk factors

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Materials and Methods

Yeast Experiments

Constructs, strains and growth conditions

The ssAβ1-42 construct consists of attB sites for Gateway cloning the Kar2 signal sequence and the Aβ1-42 sequence. The Aβ sequence was codon optimized for expression in yeast. The entire construct was synthesized and cloned into the Gateway entry vector pDONR221.

Sequence of the ssAβ construct: ACAAGTTTGTACAAAAAAGCAGGCTTCACAAA (Gateway flanking region)
ATGTTTTTCAACAGACTAAGCGCTGGCAAGCTGCTGGTACCACTCTCCGTGGTCCTGTAC GCCCTTTTCGTGGTAATATTACCTTTACAGAATTCTTTCCACTCCTCCAATGTTTTAGTT AGAGGT (Kar2 signal sequence)
GATGCTGAATTTAGACATGATTCTGGTTATGAAGTTCATCATCAAAAATTGGTTTTTTTT TGCTGAAGATGTTGGTTCTAATAAAGGTGCTATTATTGGTTTGATGGTTGGTGGTGTTG TCATTGCTTAA (Aβ1-42)

ACCCAGCTTTCTTGTACAAAGTGGT (Gateway flanking region)

The same approach was used to generate the ssAβ1-40 construct.

The BPTI WT and C51A constructs were the kind gift of Dane Wittrup (*45*). The original BPTI construct do contain a signal sequence, but we replaced it with the Kar2 signal sequence in order to target them in the same manner as Aβ. The Kar2ss sequence and Gateway flanking regions were added to the BPTI ORFs using overlap extension PCR.

The Pdi1 gene is part of the overexpression library used in the screen. The Pdi1 gene was gateway cloned into the pDONR221 entry vector. The Aβ and BPTI constructs as well as Pdi1 were cloned into the pAG426Gal vector (*46*). Constructs were transformed into W303 Mat α, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, ade2-1 using a standard lithium acetate transformation protocol.

To generate ssAβ1-42 screening strains the ssAβ1-42 construct was moved to a pAG305Gal expression vector (*46*). The plasmid was digested using BstX1, gel purified and transformed into W303. The transformation was carried out in duplicate and the level of growth of 16 transformants each was tested on synthetic deficient media lacking leucine with galactose. Two strains from the independent transformations where chosen as screening strains based on their robust yet intermediate toxicity that would allow for the identification of both suppressors and enhancers. Several transformants that showed no toxicity were selected as 1x ssAβ controls. The control strain for wild type yeast growth is carrying a Gal inducible YFP integrated in the same fashion as the ssAβ1-42 constructs.

For spotting assays strains were grown over night at 30°C in 3 mL SD media lacking the relevant amino acids and containing glucose. Cell concentrations (OD_{600})

were adjusted in a 96-well plate to that of the strain with the lowest concentration. Cells were then 5-fold serially diluted and spotted on SD media containing glucose (Uninduced) and galactose (Induced). Plates were incubated at 30°C for 2 (glucose) or 3 days (galactose).

Propidium Iodide staining

For the Propidium Iodide (PI) staining of dead yeast cells, strains were pre-grown in raffinose media over night and then induced in galactose media for 16 hr (5 mL OD_{600} 0.5). Cells were incubated with 20 μg/mL PI for 15 min, washed with PBS and then cells positive for red PI staining were counted on a flow cytometer (Guava System). 5000 cells for each sample were counted and the percent of cells that stained positive for PI were recorded. Control dead cells were generated by incubating a culture at 100°C for 5 min.

Cell lysis

Strains were grown in synthetic deficient media lacking leucine and uracil (SD-Leu-Ura) with raffinose overnight at 30°C. Cultures were the diluted into inducing media containing galactose (OD600 0.2) and grown for 8h. Cells were spun down for 5min at 3,000rpm. For preparation of yeast lysates, yeast pellets were resuspended in 200 μL of yeast lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 2.5 mM EDTA, 1% v/v Triton-X 100 with protease inhibitors). To this solution, 200 μL of glass beads (Aldrich) were added, and the yeast were bead beated for 3 minutes on maximum speed at 4°C. Afterwards, the sample was retrieved by puncturing a hole in the bottom of the eppendorf tube and spinning the samples into a new tube at 6,000 rpm for 15 seconds. The supernatant was transferred to a new tube, and this solution was used as the lysate.

Western blot protocol

For the western blot, the protein concentrations in the yeast lysates were normalized using the results from a BCA assay (Pierce). After normalization, samples were loaded on a 4-12% Bis-Tris gel (Invitrogen) and run at 150 V for approximately 50 minutes. Subsequently, the samples were transferred to 0.2 micron PVDF membrane (Bio-Rad). After the transfer was completed, the membrane was blocked with 5% milk in PBS overnight. The membrane was briefly washed with PBS before the primary antibody, 6E10 (Covance) was added at a 1:1,000 dilution in 5% milk in PBS. The primary antibody was allowed to incubate for 2.5 hours, after which the blot was washed 4 x 5 minutes with PBS. The anti-mouse secondary (DyLight, Rockland) was added at a 1:10,000 dilution in 5% milk in PBS for 1 hour. The blot was washed 4 x 5 minutes with PBS. The blot was scanned using the Licor Odyssey Scanner.

For detection of oligomers using the NAB61 antibody, the antibody was diluted 1:500 in 5% milk in PBS and incubated with the blot. Subsequently, the blot was washed 4 x 5 minutes with PBS. The anti-mouse secondary (DyLight, Rockland) was

added at a 1:10,000 dilution in 5% milk in PBS for 1 hour. The blot was washed 4 x 5 minutes with PBS. The blot was scanned using the Licor Odyssey Scanner.

Indirect ELISA protocol

The indirect ELISA using the OMAB oligomer specific antibody was performed as described in Lindhagen-Persson *et al*. (*47*). Briefly, the oligomer specific monoclonal OMAB antibody (Agrisera AB, Vännäs, Sweden) was diluted to a concentration corresponding to 5 μg/mL and was coated to a 96-well polystyrene microtiterplate (F96 Nunc immunosorp, Denmark) overnight. Unspecific sites were blocked using 5% dry milk dissolved in PBS. Frozen yeast lysates were thawed on ice and centrifuged at 20,000 x g for 5 minutes followed 3 serial dilutions in PBS. The plate coated with OMAB antibody was washed with water and 100 μl of the samples was applied in 5 replicates. The samples were incubated at room temp for 25 minutes, and the plate was subsequently washed 4 x with PBS containing 0.15% Tween-20. Bound Aβ was detected using a polyclonal rabbit anti-Aβ antibody (antibody AS08 357, Agrisera AB, Vännäs Umeå) that was dissolved in blocking buffer at a 1:2,000 dilution. The rabbit antibody was allowed to incubate for 30 minutes, after which the plate was washed with PBS containing 0.15% Tween-20. A secondary anti-rabbit IgG-HRP (GE-healthcare, Buckinghamshar, UK) dissolved in blocking buffer was used to detect bound rabbit IgG. Before addition of the detection reagent, excess secondary anti-rabbit IgG-HRP was removed by washing with PBS containing 0.15% Tween-20. The plate was developed using 100 μL EC-blue® (Medicago, Uppsala, Sweden). Measurements were made at 400 nm and 600 nm, and the absorption ratio between 600 nm and 400 nm was used to calculate the level of binding. Statistical significance was determined using the paired *t*-test. For the lysate plus monomeric Aβ sample, monomeric Aβ was added to vector control cells that were being lysed. These samples were analyzed in the OMAB ELISA assay. No signal was detected in the samples with the monomeric Aβ peptide, supporting that the assay does not detect monomeric Aβ, and that our lysis conditions do not induce Aβ oligomerization.

Size exclusion chromatography was used to analyze the relative sizes of oligomers formed in our yeast ssAβ 1-42 screening strains. Lysates of the ssAβ 1-42 screening strains were separated on a superose-12 HR column 20 mL PBS. Fractions were collected and each fraction was analyzed in triplicate using the OMAB antibody in an indirect ELISA assay. To determine the molecular weight of each fraction, a standard of known proteins (Biorad 151-1901) was used as well as a single run of monomeric Aβ 1-42. Under these chromatography conditions, monomeric Aβ 1-42 migrates with an apparent molecular weight of about 11 kDa. Dimers and tetramers also have this exclusion volume. Upon an increase in size of the oligomeric Aβ species, the "non-ideal behavior" diminishes and Aβ behaves as a normal globular protein.

Immunostaining

Strains were pre-grown in raffinose media over night and then induced in galactose media for 16 hr (5 mL OD_{600} 0.5). Cells were spun down and resuspended in 5 mL 4% formaldehyde, 50mM KP_i, pH 7.4, 1mM $MgCl_2$. Cells were fixed for 2 hours. After fixation, cells were washed 3 times in 5 mL of wash buffer (0.1M KP_i pH 7.4, 1mM $MgCl_2$, Complete Mini Protease Inhibitors (Roche)) and then resuspended to a final OD600 of 10 in the wash buffer. 100μl of cells were incubated with 0.6 μL of 2-mercaptoethanol and 20 μL of yeast lytic enzyme (25,000U/ml; ICN) for 10min and then washed and gently resuspended in 100 μL wash buffer. Ten μL of the spheroplast suspension was added to a polylysine-coated coverglass, incubated for 3 min and then blotted dry. The dried coverglass is submerged in 40 mL of acetone precooled to -20°C in a 50 mL Falcon tube. After 5min, the coverglass is removed, inverted onto a paper towel and allowed to dry. Each well was pre-coated for 30 min with PBS-Block (PBS, pH7.4, 1% dried milk, 0.1% bovine serum albumin, 0.1% octyl glucoside). Block was gently aspirated off and 10 μL of the primary antibody mixture (PBS-Block, 1:200 6E10 Aβ antibody) was added. Following 1hr incubation, the primary antibody was gently washed off 8 times with PBS-Block. Ten μL of the secondary antibody mixture (PBS-Block, 1:100 anti-mouse FITC, 2μg/ml Hoechst 33258) was added, incubated for 30min in the dark and washed off 8 times. The liquid is aspirated completely after the final wash. 5μl of mounting medium is added to each well before it is placed on a slide and sealed with nail polish.

Images were taken on a Nikon Eclipse Ti inverted microscope using a Roper Scientific CoolSNAP HQ camera. Z-stacks were taken at a distance of 0.3 μM between slices and the NIS Elements Microscope Imaging Software was used to deconvolve and process the images.

Sample preparation for mass spectrometry

Control and ssAβ 1-42 strains were induced, lysed and subsequently immunoprecipitated with the 6E10 antibody. The immunoprecipitated material was subjected to SDS-PAGE and silver staining. The ~3.5kd and 6kd region of the control and ssAβ screening strain lanes were excised. These bands of interest were divided into ~2 mm squares and washed overnight in 50% methanol/ water. These were washed once more with 47.5/47.5/5 % methanol/water/acetic acid for 2 hours, dehydrated with acetonitrile and dried in a speed-vac. Reduction and alkylation of disulfide bonds was then carried out by the addition of 30 ul 10 mM dithiothreitol (DTT) in 100 mM ammonium bicarbonate for 30 minutes to reduce disulfide bonds. The resulting free cysteine residues were subjected to an alkylation reaction by removal of the DTT solution and the addition of 100 mM iodoacetamide in 100 mM ammonium bicarbonate for 30 minutes to form carbamidomethyl cysteine. These were then washed with aliquots of acetonitrile, 100 mM ammonium bicarbonate and acetonitrile and dried in a speed-vac. The bands were enzymatically digested by the addition of 300 ng of trypsin in 50 mM ammonium bicarbonate to the dried gel pieces ratio of mg trypsin for 10 minutes on ice. Depending on the volume of acrylamide, excess ammonium bicarbonate was removed or enough was added

rehydrate the gel pieces. These were allowed to digest overnight at 37°C. The resulting peptides were extracted by the addition of 50 μL (or more if needed to produce supernatant) of 50 mM ammonium bicarbonate with gentle shaking for 10 minutes. The supernatant from this was collected in a 0.5 mL conical autosampler vial. Two subsequent additions of 47.5/47/5/5 acetonitrile/water/formic acid with gentle shaking for 10 minutes were performed with the supernatant added to the 0.5 mL autosampler vial. Organic solvent was removed and the volumes were reduced by to 15 μL using a speed vac for subsequent analyses.

Chromatographic separations

Digestion extracts were analyzed by reversed phase high performance liquid chromatography (HPLC) using a Waters NanoAcquity HPLC and autosampler and a ThermoFisher LTQ linear ion trap mass spectrometer using a nano flow configuration. A 20 mm x 180 micron column packed with 5 micron Symmetry C18 material (Waters) using a flow rate of 15 μL per minute for two minutes was used to trap and wash peptides. These were then eluted onto the analytical column which was a self-packed with 3 micron Jupiter C18 material (Phenomenex) in a fritted 10 cm x 75 micron fused silica tubing pulled to a 5 micron tip. The gradient was isocratic 1% A Buffer for 1 minute 250 nL min-1 with increasing B buffer concentrations to 40% B at 20 minutes. The column was washed with high percent B and re-equlibrated between analytical runs for a total cycle time of approximately 37 minutes. Buffer A consisted of 1% formic acid in water and buffer B consisted of 1% formic acid in acetonitrile. (Alternatively, a linear gradient of 1% to 40% B from time 1 min to time 24.5 minutes is used as an alternative for very low level samples where staining indicates a single band.)

Mass spectrometry of isolated proteins

The ThermoFisher LTQ linear ion trap mass spectrometer was operated in a dependant data acquisition mode where the five most abundant peptides detected in full scan mode were subjected to daughter ion fragmentation. A running list of parent ions was tabulated as an exclusion list to increase the number of peptides analyzed throughout the chromatographic run.

Mass spectrometry data analysis

Peptides were identified from the MS data using SEQUEST algorithms that searched a species-specific database generated from NCBI's non-redundant (nr.fasta) database. Sequest filters used for indication of a positive peptide identification were: XCorr vs. Charge State = 1.5, 2.00, 2.50; Sp – Preliminary Score = 500. Two peptides were required for a protein to be considered a positive identification. Data interpretation from all bands was aided by the MSRAT program (Protein Forest) or Scaffold (Proteome Software).

Halo secretion assay

The α-syn control strain used in this assay was Mata, 303 Gal α-syn YFP, 304 Gal α-syn YFP, leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15. Tested strains were struck onto a YPD plate (w/v – 1% yeast extract, 2% peptone, 2% glucose,

adjusted to pH 7.0, 2% agar) and grown overnight at 30°C. The next day bar1 cells were grown for 4 hours in YPD (w/v – 1% yeast extract, 2% peptone, 2% glucose, adjusted to pH 7.0) before being diluted 1:50 and plated as a lawn on both YPD and YPGal (w/v – 1% yeast extract, 2% peptone, 2% glucose, adjusted to pH 7.0, 2% agar) plates. After the lawns dried, the strains to test were scrapped from the plate and diluted into sterile water. For uninduced controls, 10 μL of culture diluted to an OD_{600}=1.0 was spotted in the center of the YPD plate. For the inducing YPGal plates, an OD_{600}=1.0 was used for α-syn and the toxic Aβ screening strain 1, while an OD_{600}=0.04 was used for the vector control strain to account for the difference in rate of growth. Pictures were taken after two days of growth at 30°C.

Screen for modifiers of Aβ toxicity

The overexpression library screened contains ~5800 full-length sequence verified yeast ORFs in the galactose-inducible Gateway expression plasmid pBY011 (*CEN*, *URA3*, AmpR) (*48*). The library is arrayed in 96-well format. Plasmid DNA was prepared by pin inoculation into deep well 96-well plates containing 1.8mL LB-AMP, growth over night at 37°C and 96-well mini preps using a Qiagen BioRobot 8000. The DNA was transformed into a ssAβ screening strain (ssAβ1-42 p305) carrying a Gal4-ER-VP16 plasmid (*CEN*, *HIS3*, AmpR), which allows for expression from GAL promoters on carbon sources other than galactose in the presence of estradiol in the yeast media (*49*). Neither estradiol nor the Gal4-ER-VP16 plasmid had an effect on Aβ toxicity on its own. Transformations were carried out using a standard lithium acetate transformation protocol adapted for a 96-well format and automation using a Tecan Evo 150 liquid handling robot. Transformants were grown in synthetic deficient media lacking histidine, leucine, and uracil (SD-His-Leu-Ura) with glucose overnight. The cells were then diluted in water and spotted on SD-His-Leu-Ura agar plates containing glucose alone (control), galactose alone, glucose plus 1μM estradiol (Sigma E1024) or glycerol plus 1μM estradiol using a Singer RoToR pinning robot and long 96-well pins. Putative enhancers and suppressors were identified after 2-4 days of growth at 30°C. Putative screen hits were cherry picked from the plasmid library, retransformed into two independent derived ssAβ screening strains and retested on the three screening conditions in two independently derived strains. We eliminated hits that have known effects on GAL induction and genes whose overexpression has previously been shown to be toxic. To further exclude false-positive suppressors we used flow cytometry to measure the expression of YFP in their presence. To further exclude false-positive enhancers that cause a general inhibition when overexpressed we examined their effects in the YFP control strain, which has no growth impairment. The identity of confirmed modifiers was verified by sequencing.

Flow cytometry

A strain carrying an integrated YFP was transformed with the putative Aβ suppressors. The resulting strains were grown in glucose media in a 96-well format, diluted into the various inducing media (galactose, glucose +1μM estradiol, glycerol + 1μM estradiol) [5 μL culture added to 120 μL media], and incubated over night at

30°C with mild shaking. These (*50*) overnight cultures were diluted 20-fold into water and YFP levels were measured using a Guava flow cytometer. Each strain was measured 3 times and 5000 cells were counted for each well. The whole experiment was repeated 3 times. Values are averages of these 3 experiments and reported in percent of the vector control strain YFP levels.

Microscopy of Clc1-GFP and Ste3-YFP

We created our own version of the Ste3 localization assay (*53*) by generating a *GPD*-driven Ste3-YFP construct, using the Ste3 plasmid from our ORF library and a *GPD* p303 vector from the pAG collection (*46*), and integrating it into an ssAβ 1-42 screening strain as well as a control strain. We tested the effect of selected modifiers by transforming these strains with the modifiers and analyzing them in the same fashion. For all microscopy experiments strain were pre-cultured in raffinose media and then induced in galactose media for 16 hr. Images were taken on a Nikon Eclipse Ti inverted microscope using a Roper Scientific CoolSNAP HQ camera. Z-stacks were taken at a distance of 0.3 μM between slices and the NIS Elements Microscope Imaging Software was used to deconvolve and process the images. The intensity of the control Ste3-YFP strain was significantly brighter than the cells expressing Aβ, so we had to increase the exposure time in order to see an interpretable signal. The control strain was imaged for 230ms, while the Aβ-expressing strains were all exposed for 1.15s. Aside from the difference in exposure time, we processed all of the samples exactly the same.

To examine the effects of Aβ on endocytosis, we mated the Aβ screening strains to the clathrin light chain (Clc1)-GFP strain from the GFP library (*54*). We also used GFP-fusion strain of other endocytic proteins (Abp1, Sla1 and Sla2) and observed similar Aβ-induced changes in localization as with Clc1-GFP (data not shown); yet the fluorescence of these fusions was rather low. Images were captured and processed as described above.

C. elegans Experiments

Plasmid & Constructs

The following cDNAs were cloned into pDONR221 using Gateway Technology (Invitrogen, San Diego, CA): *ssAβ 1-42* (Chris Link, University of Colorado, for the signal sequence we used Chris Link's *C. elegans* signal sequence: ATGCATAAGGTTTTGCTGGCACTGTTCTTTATCTTTCTGGCACCAGCAGGTACC); *gfp* and *lacZ* (Andy Fire, Stanford University); *mCherry*; *C13G3.3*, *C32E8.10*, and *C37H5.6* (Worm ORFeome collection from Marc Vidal) (*55*); *XPO1* (human ORFeome collection from Marc Vidal) (*56*); and *JC8.10*, *Y44E3A.4*, and *F42G10.2*. *JC8.10*, *Y44E3A.4*, and *F42G10.2* were isolated from our *C. elegans* cDNA library. The cDNAs were verified by DNA sequencing, and subsequently cloned into pDEST-EAT-4. pDEST-EAT-4 was generated by PCR amplification of a *eat-4* promoter, double

digestion of the promoter and pDEST-UNC-54 using *BplI* and *KpnI*, and replacement of a *unc-54* promoter in pDEST-UNC-54 with a *eat-4* promoter via ligation reaction.

Nematode Strains

Nematodes were maintained following standard procedures(*57*). To make a worm ssAβ 1-42 model UA162 [*baEx*107; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry], 50 μg/mL of P*eat-4*::ssAβ 1-42 and P*eat-4*::gfp as well as 2.5 μg/mL of P*myo-2*::mCherry were injected into wildtype N2 (Bristol) worms. This strain was integrated by using Spectrolinker XL-1500 (Spectronics Corporation, Westbury, NY) and outcrossed three times to N2 worms to generate UA166 [*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]. For neuroprotection analysis, three stable lines of UA163 [*baEx*108; [P*eat-4*::C13G3.3, rol-6 (su1006)], UA164 [*baEx*109; [P*eat-4*::C32E8.10, rol-6 (su1006)], and UA165 [*baEx*110; [P*eat-4*::C37H5.6, rol-6 (su1006)] were made and crossed with UA166 [*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry] to generate UA167 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*108; [P*eat-4*::C13G3.3, rol-6 (su1006)]}, UA168 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*109; [P*eat-4*::C32E8.10, rol-6 (su1006)]}, and UA169 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*110; [P*eat-4*::C37H5.6, rol-6 (su1006)]}. Furthermore, three stable lines of UA170 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*111; [P*eat-4*::JC8.10, rol-6 (su1006)]}, UA171 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*112; [P*eat-4*::Y44E3A.4, rol-6 (su1006)]}, UA172 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*113; [P*eat-4*::XPO1, rol-6 (su1006)]}, UA173 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*114; [P*eat-4*::F42G10.2, rol-6 (su1006)]}, UA174 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*115; [P*eat-4*::mCherry, rol-6 (su1006)]}, and UA175 {[*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry]; *baEx*116; [P*eat-4*::lacZ, rol-6 (su1006)]} were generated by directly injecting 50 μg/mL of putative ssAβ 1-42 toxicity modifiers and *rol-6* into UA166 [*baInl*32; P*eat-4*::ssAβ 1-42, P*eat-4*::gfp, P*myo-2*::mCherry].

Neuroprotection Analysis

For analysis of putative ssAβ 1-42 toxicity modifiers, the transgenic worms were age-synchronized (*58*), transferred onto NGM plates, and grown at 20°C for 3 or 7 days. For each trial, 30 worms were transferred to a 2% agarose pad, immobilized with 2mM levamisole, and scored. Worms were considered rescued when all five posterior glutamatergic neurons were intact and had no visible signs of degeneration. Each stable line was analyzed three times (for a total of 90 worms/transgenic line). Three separate transgenic lines were analyzed per gene (for a total of 270 animals/gene). Imaging and statistics were performed as described previously (*59*).

Semi-quantitative RT-PCR

RNA isolation and semi-quantitative RT-PCR were performed as described previously (*59*). Briefly, total RNAs were isolated from 50 L3-staged worms,

amplified using SuperScript III RT (Invitrogen) with oligo dT primers, and treated with amplification grade RNase-free DNase I (Invitrogen) as well as RNase H (Invitrogen) following the manufacture's protocol. The following primers were designed for the PCR:

cdk-5	Primer 1:	5' ggg-gat-gat-gag-ggt-gtt-cca-agc 3'
	Primer 2:	5' ggc-gac-cgg-cat-ttg-aga-tct-ctg-c 3'

The trangenes were PCR amplified by using primer sequences specific to *unc-54* 3'UTR and each respective open reading frame.

unc-54 3'UTR	Primer 1:	5' gac-tta-gaa-gtc-aga-ggc-acg-ggc 3'
ssAβ 1-42	Primer 2:	5' atg-cat-aag-gtt-ttg-ctg-gca-ctg-ttc-ttt-atc 3'
C13G3.3	Primer 2:	5' gag-aaa-cag-gca-atg-gga-aac-ccg-c 3'
C32E8.10	Primer 2:	5' gct-gct-cca-ttc-gga-tat-cca-aat-gc 3'
C37H5.6	Primer 2:	5' gga-gta-acg-act-gga-cgt-aaa-cgt-cg 3'
JC8.10	Primer 2:	5' gat-cga-cct-cgt-cca-cca-tca-gc 3'
Y44E3A.4	Primer 2:	5' cac-tga-tca-ggt-cgc-cga-act-gc 3'
F42G10.2	Primer 2:	5' cat-gac-gcc-ggt-tgt-cag-ccg 3'
XPO1	Primer 2:	5' gtg-aca-gac-act-tca-cat-act-gct-gg 3'
mCherry	Primer 2:	5' gat-gaa-ctt-cga-gga-cgg-cgg-c 3'
lacZ	Primer 2:	5' gcc-tta-ctg-ccg-cct-gtt-ttg-acc 3'

Cortical Neuron Experiments

Soluble Aβ oligomer and Aβ fiber preparations

Soluble oligomers were prepared as in Kayed *et al. (51)*. In brief, lyophilized Aβ 1-42 (American Peptide Company) was resuspended in 200μL of HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol, Aldrich), and this solution was bath sonicated for 30 minutes. Subsequently, 100 μL of the HFIP solution was added to 900μL of ddH2O in a siliconized eppendorf tube. This solution was incubated at room temperature for 10-20 minutes. After the room temperature incubation, the HFIP in the sample was evaporated using a gentle stream of N_2 for approximately 20 minutes. The samples were allowed to incubate at room temperature for 48 hours, after which they were frozen and stored at -80°C. Oligomers were characterized by SDS-PAGE analysis as described above and by SDD-AGE as described in Halfmann and Lindquist (*50*). Fibers were prepared by dissolving 0.5 mg of Aβ 1-42 (American Peptide Company) in 200 μL HFIP. The resulting solution was bath sonicated for 30 minutes at room temperature. The resulting disaggregated Aβ sample was dried to a peptide film using a gentle stream of N_2. Subsequently, the peptide film was resuspended in 93.75 μL of DMSO, and to this solution, 1300 μL of 10mM HCl was added. The samples were incubated at 37°C for several days without shaking. Fibers were characterized using SDD-AGE. Electron Microscopy was performed at the W.M. Keck Microscopy Facility at the Whitehead Institute. For

preparation of the EM samples, the fibers were adhered to a copper surface and stained with 2% uranyl acetate. The fibers were imaged using a FEI Technai Spirit Transmission Electron Microscope.

Rat primary cortical cultures

Cultures were prepared based on Lesuisse and Martin (*52*). Embryos were harvested by cesarean section from anesthetized pregnant Sprague-Dawley rats at embryonic day 18. Cerebral cortices were isolated and dissociated with Accumax digestion for 20 min at 37°C and trituration with Pasteur pipette. Poly-ornithine and lamine-coated 96-well plates were seeded with $4x10^4$ cells in neurobasal medium (Life Technologies) supplemented with B27 (Life Technologies), 0.5mM glutamine, 25μM β–mercaptoethanol, penicillin (100 IU/mL) and streptomycin (100μg/mL). One third of the medium was changed every 3 to 4 days. Aβ oligomer (final concentration 750nM) or vehicle was added to the lentivirus-transduced cultures in 96-well plates at DIV 18. As a surrogate marker of cell viability, cellular ATP content was measured after 20 hours of Aβ oligomer incubation using ViaLight Plus kit (Lonza). As a secondary method for quantitating neuronal toxicity, neurons were stained with an antibody specific for the neuron MAP-2 (see details below).

Lentivirus production and transduction to rat primary cortical cultures

pLENTI6/V5 DEST (Invitrogen) lentivirus expression vector was used to generate lentivirus encoding *GFP*, *PICALM* and *RAB1*. Lentiviral constructs were packaged into virus via lipid-mediated transient transfection of the expression constructs and packaging plasmids (pMD2.G and psPAX2) to 293 cells. Lentivirus was purified and concentrated using Lenti-X Maxi Purification kit and LentiX Concentrator (Clontech) according to the manufacturer's protocol. Lentivirus titer was determined using QuickTiter Lentivirus titer kit (Lentivirus- Associated HIV p24; Cell Biolabs) according to the manufacturer's protocol. Rat cortical cultures were transduced with various multiplicities of infection (MOI) of lentivirus at DIV 5.

Immunocytochemistry and quantification of viable neurons.

For imaging, cells were cultured in PerkinElmer View plates-96F TC (Waltham, MA), coated with poly-ornithine / laminin. For immunohystochemical staining, cells were rinsed with PBS, fixed in 4% paraformaldehyde for 20 min, permeabilized and blocked for 1 h in blocking buffer (PBS, containing 0.1% Triton X100 and10% normal donkey serum). Cells were then incubated with rabbit polyclonal antibodies against MAP-2 (1:500, Millipore, Temecula, CA) in blocking buffer at 4°C overnight. After the primary antibody incubation, cells were rinsed with PBS and incubated with secondary antibodies (Alexa Fluor 568 donkey anti-rabbit IgG (1:500, Invitrogen) for 1 hour at room temperature. After rinsed three times with PBS, nuclei were stained with Hoechst 33342 (Invitrogen) for 15 min and rinsed with PBS. Images were taken using an Eclipse Ti Nikon microscope (10x objective). The number of live neurons was determined by manually counting MAP-2 and Hoechst-positive cells from random 20 fields per well, 3 wells per condition.

Analysis of ROS and MAP data sets

We leveraged available genotyping, and extensive clinical and pathological data from two large epidemiological studies of aging, cognition, and AD: the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP). These studies enlisted more than 2,300 older persons, without dementia at baseline, who were clinically evaluated annually and who agreed to brain donation upon death. Nearly 900 autopsies have been completed. Recent studies in these cohorts demonstrate how intermediate AD-related cognitive and pathological phenotypes can enhance power for genetic association analysis (*60-62*). For our study, we utilized a combined cohort of 1,593 ROS and MAP subjects with longitudinal neuropsychiatric assessments and genome-wide genotyping, and a nested pathological cohort including 651 brain autopsies (Table S4).

We initially determined whether the modifiers correspond to loci that impact susceptibility for episodic memory decline, a cardinal feature of AD. In ROS and MAP, rate-of-change in memory performance is characterized based on repeated assessment of 7 neuropsychiatric tests, and our analyses were additionally adjusted for age, gender and years of education. We implemented a locus-based association test for memory decline, considering all common single nucleotide polymorphisms (SNPs) at each candidate locus, including both directly genotyped and imputed variants, based on the HapMap reference (*63*) (Table S5 & S6). We tested if the observed associations were significant, by performing a permutation procedure to compute an empirical *P*-value (P_{perm}), adjusting for the multiple tests performed at each locus. Aside from *PICALM* (*rs7128598*, *p-value*=1.6x10^{-4}, P_{perm}=0.012), several other loci harbored SNPs suggesting association with memory decline, but these results did not remain significant following permutation (Table S6).

We investigated whether our modifiers are associated with the development of AD neuropathology. These analyses used a quantitative summary measure of global AD pathologic burden, based on counts of amyloid plaques and NFTs on brain tissue sections. The relations of SNPs with this continuous measure of pathology were tested using linear regression, adjusting for age at the time of death. Notably, these analyses indicate association of 2 additional loci identified by our yeast screen, *ADSSL1* (*rs1128880*, *P*=0.001, *Pperm*=0.031) and *RABGEF1* (*rs17566701*, *P*=0.002, *Pperm*=0.038) with AD neuropathology (Table S7). Both of these loci also harbored suggestive association signals with episodic memory decline in the larger clinical cohort, showing a statistical trend toward significance following the permutation procedure (Table S6).

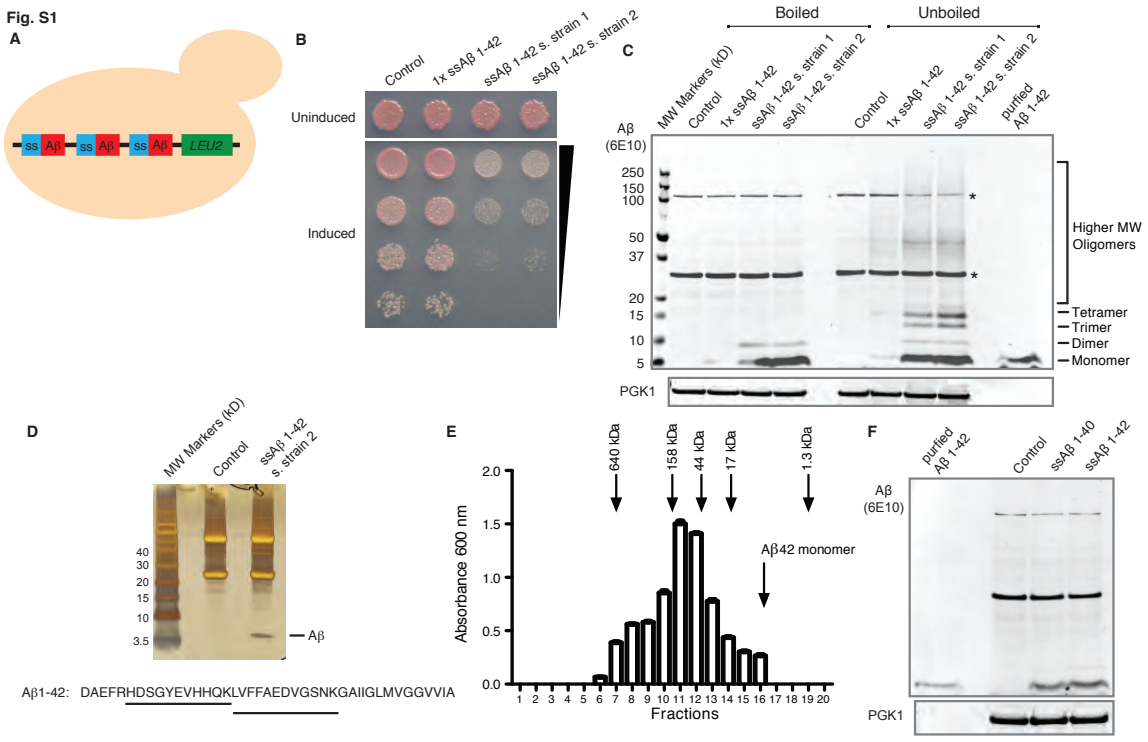

Fig. S1. Characterization of strains expressing Aβ

(A) The genetic screening strains were generated by integrating multiple tandem copies of the ssAβ 1-42 construct into the *LEU2* locus of W303 strains (see Materials and Methods). (B) Construction of two independent stable ssAβ 1-42 strains for a genetic screen. A single integrated genomic copy of ssAβ 1-42 (1 x ssAβ 1-42) was not toxic, but the integration of multiple copies of the construct resulted in robust toxicity. (C) Aβ 1-42 expression in the vector control, 1 x ssAβ and two ssAβ screening strains was detected by immunoblot analysis using the 6E10 Aβ-specific antibody. Samples were loaded onto a Bis-Tris gel with and without prior boiling in LDS sample buffer. We detected higher molecular weight species of Aβ corresponding to trimers, tetramers and higher molecular weight oligomeric species only in the unboiled Aβ 1-42 samples, indicating that these species are indeed non-covalent oligomeric assemblies of Aβ. Asterisks (*) refer to non-specific reactivity of the antibody, as these proteins are also present in the vector control lysate. (D) Mass spectrometry of immunoprecipitated material from Aβ screening strain. Extracts of a vector control and a ssAβ 1-42 screening strain were immunoprecipitated with the 6E10 antibody and this material was subjected to SDS-PAGE and silver staining. The ~3.5 kDa and 6 kDa region of each the control lane and the Aβ screening strain lane were excised. Proteins and peptides in these slices were eluted from the gel, subjected to tryptic digestion, and subjected to mass spectrometric analysis. The sequence of the human Aβ 1-42 peptide is shown and the sequence regions that are underlined are the sequences of the two tryptic peptides detected only in the ssAβ cells by mass spectrometry (HDSGYEVHHQK and LVFFAEDVGSNK). No other peptides were detected in either strain. (E) Lysate of ssAβ 1-42 screening strain 2

was fractionated using size exclusion chromatography. Individual fractions collected from the size exclusion column were individually assayed using an indirect ELISA assay with a monoclonal Aβ oligomer-specific antibody (referred to as OMAB). Molecular weights corresponding to fractions 7, 10, 12, 14 and 19 were calculated based on protein standards run on the same column using identical conditions. Data are representative of three independent experiments and shown as mean +/- SEM. (F) Aβ 1-40 and Aβ 1-42 expression was detected by immunoblot analysis of boiled lysates using 6E10.

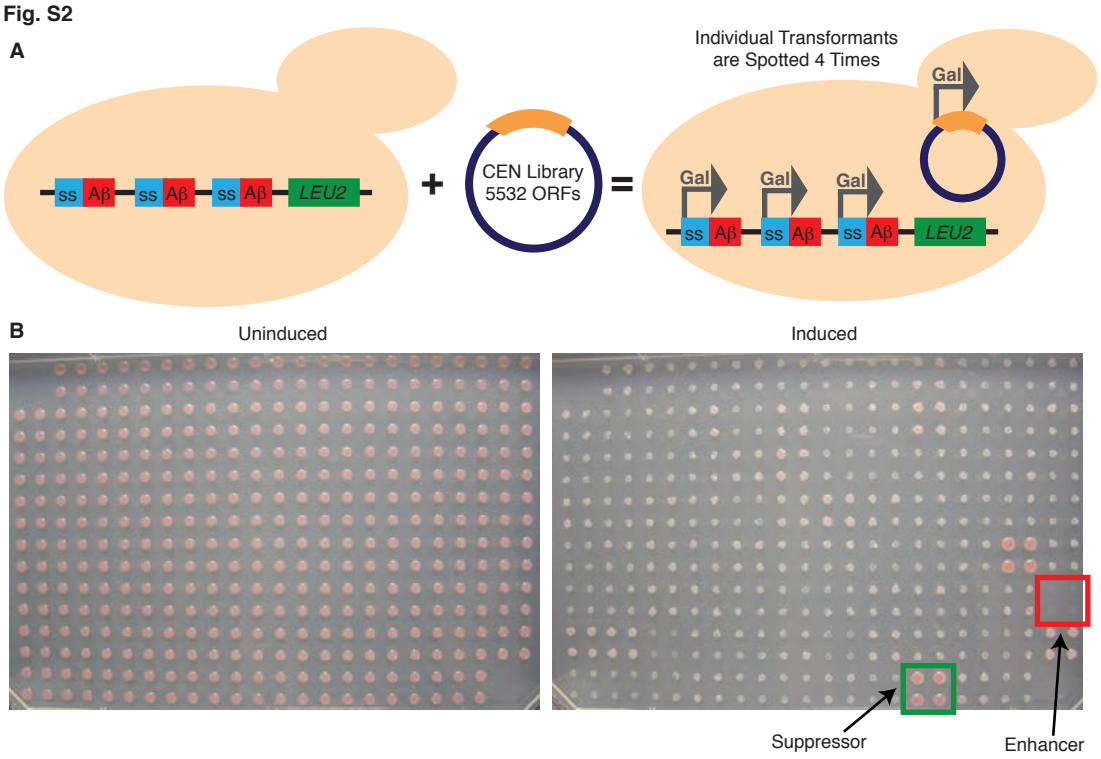

Fig. S2: Example of a screening plate
(A) Yeast strains carrying the ssAβ 1-42 construct and the Gal4-Estrogen Receptor (ER)-VP16 transcription factor were transformed with a CEN plasmid library containing a genome-wide collection of ORFs. (B) Each transformant was spotted 4 times in a square array using a Singer RoToR robot on media containing galactose, glucose and estradiol, glycerol and estradiol, or glucose alone as a non-inducing growth control. Enhancers resulted in decreased growth and suppressors in increased growth; refer to boxed examples. Empty quadrants on the plate are the result of empty wells in the plasmid library.

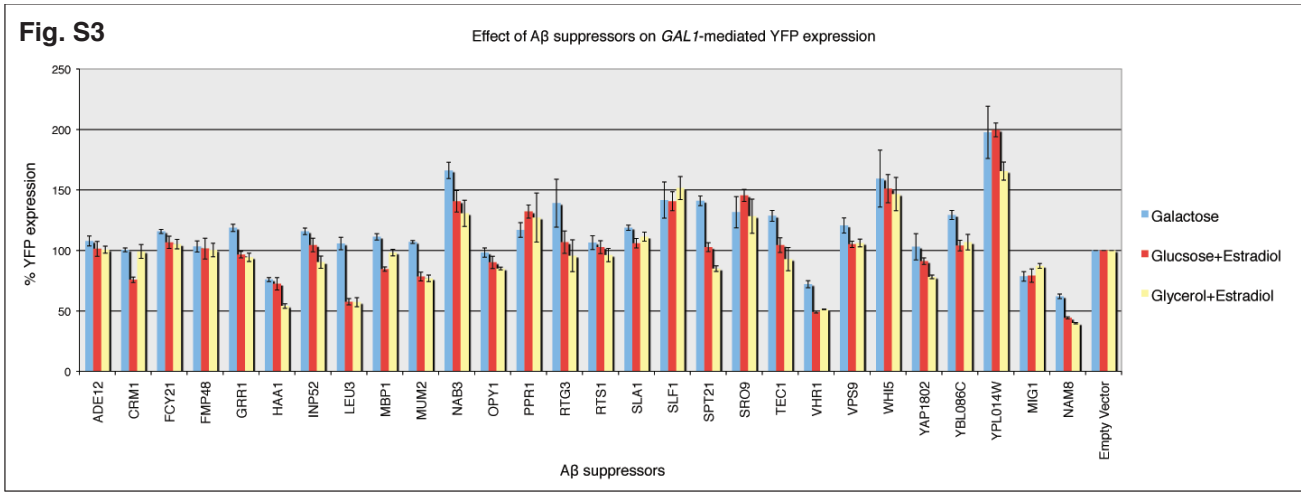

Fig. S3: Effect of suppressors on YFP expression levels
Putative suppressors may decrease Aβ toxicity by decreasing GAL1-mediated expression. We assessed whether any of the identified suppressors affected GAL1-mediated expression by assaying the expression of Yellow Fluorescent Protein (YFP) under the control of a GAL1 promoter. Putative suppressors were transformed into a strain carrying an integrated GAL1-controlled YFP construct and the Gal4-ER-VP16 transcription factor. The effect of putative hits on YFP expression levels was quantified using flow cytometry after overnight growth in one of three media conditions: galactose, glucose with estradiol or glycerol with estradiol. Hits that significantly decreased the levels of YFP in comparison to the vector control were eliminated as false positives (i.e. HAA1, LEU3 and VHR1). We did not eliminate suppressors that showed activity on all three media conditions, even if YFP expression levels were lower for one of the growth conditions.

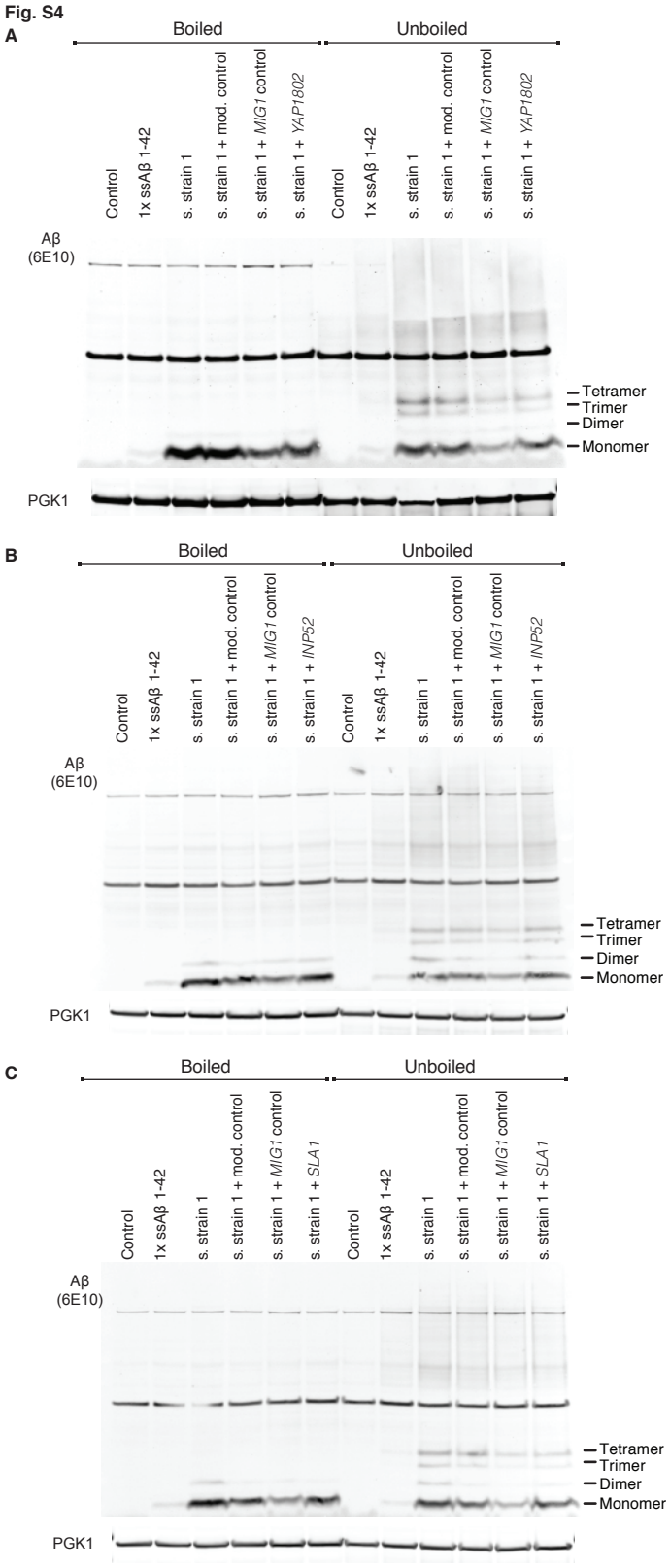

Fig. S4: Effect of *YAP1802, INP52,* and *SLA1* on Aβ levels.
We transformed the ssAβ 1-42 screening strain 1 with either a modifier (mod.) vector control, a *MIG1* control, or one of the following suppressors: (A) *YAP1802,* (B)

INP52, or (C) *SLA1*. The levels of Aβ 1–42 in each of these strains were assessed by western blot analysis with the 6E10 antibody. As expected, *MIG1*, which suppresses *GAL1*-mediated transcription, decreased levels of Aβ 1-42 expression, as compared to the vector control. The yeast expressing either (A) *YAP1802*, (B) *INP52,* or (C) *SLA1* showed a small decrease in the amount of low molecular weight oligomers as compared to the modifier vector control. The overall amount of Aβ 1-42 in the yeast expressing (A) *YAP1802,* (B) *INP52,* or (C) *SLA1* are remarkably similar to the level observed in the control lanes (refer to the boiled samples). These data suggest that these three modifiers induce only subtle changes in Aβ levels.

Fig. S5

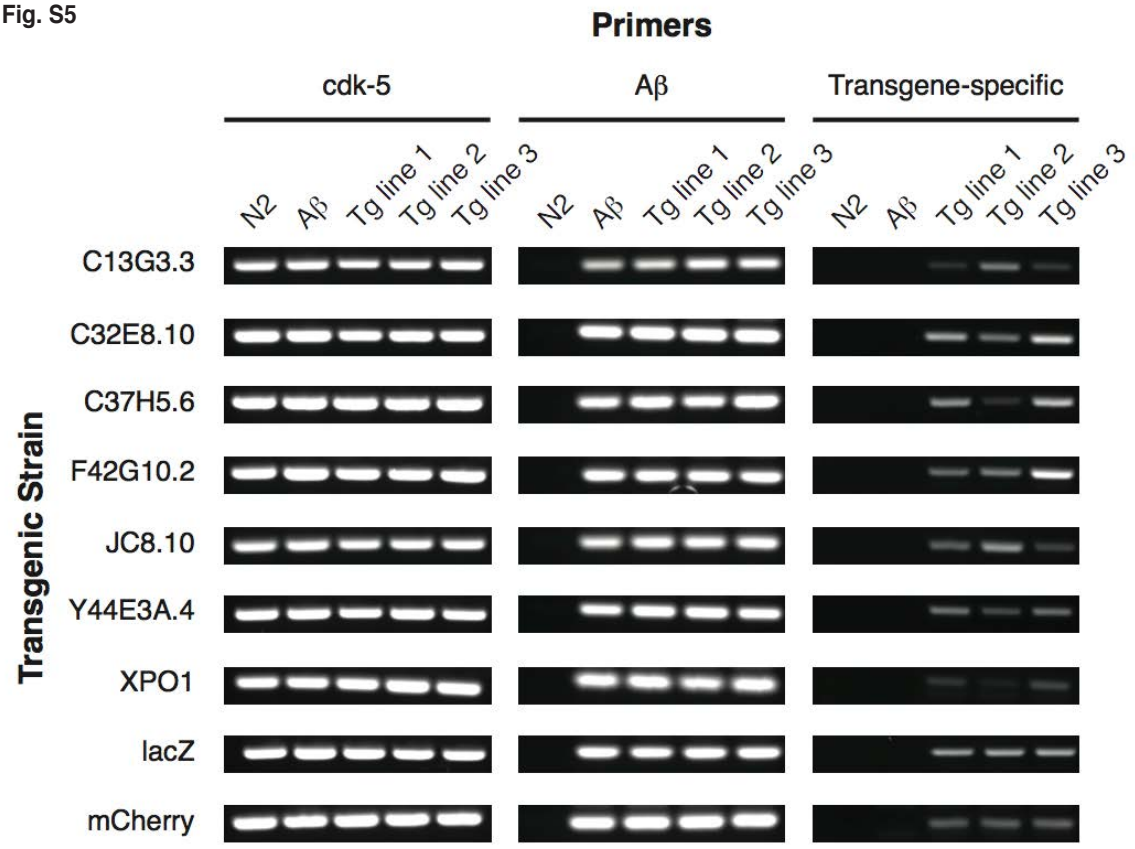

Fig. S5: Analysis of transgene expression in worm strains.
We conducted semi-quantitative RT-PCR to ensure that transgenes did not influence ssAβ 1-42 expression in *C. elegans*. We could not measure Aβ levels by western blot analysis as we expressed Aβ in only specific cells per animal. The PCR was conducted by using primers designed to amplify *cdk-5* (control), Aβ, and indicated transgenic modifiers of Aβ induced neurodegeneration. For all primers, a N2 wildtype strain served as both a positive (*cdk-5*) and negative (Aβ and transgenes) control. The ssAβ transgene of the Aβ strain (UA166) was integrated into worm chromosomal DNA to maintain steady Aβ expression level in the entire organism (see Materials and Methods). Using UA166 strain, 9 additional transgenic strains expressing homologs of Aβ toxicity modifiers identified in the yeast genetic screen were generated. Expression levels of the corresponding modifiers varied since the transgenes remained as extrachromosomal arrays. To address the discrepancies, we generated 3 independent lines for each of the 9 transgenic strains. Importantly, co-expression of the modifiers did not change the ssAβ expression level.

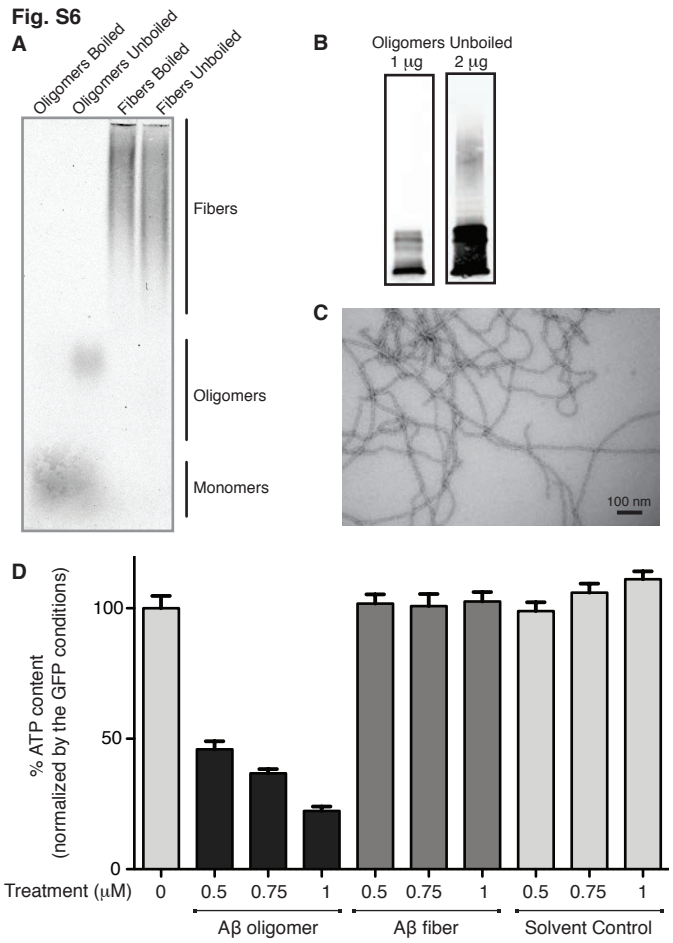

Fig. S6: Characterization of Aβ oligomers and Aβ fibers used in cortical neuron experiments.

(A) SDD-AGE characterization of Aβ 1-42 oligomers and Aβ 1-42 fibers prepared from synthetic peptide. Oligomer and fiber samples were either not boiled or boiled in 6% sarkosyl buffer. These samples were subsequently run on a 1.2% SDD-AGE gel. Upon boiling, Aβ 1–42 oligomers collapsed into monomeric species, while unboiled oligomers ran as species of intermediate size on the SDD-AGE gel. Fiber samples were resistant to boiling, consistent with the formation of an SDS-resistant amyloid structure. (B) Unboiled Aβ 1-42 oligomers were also characterized by western blot using the 6E10 antibody. These samples contained a diversity of low and high molecular weight oligomeric species. (C) Aβ fiber formation was also detected by negative stain Electron Microscopy (EM). The scale bar is representative of 100 nm. (D) Cortical neuron cultures prepared from rat embryos at embryonic day 18 were cultured for 5 days, and were subsequently incubated for 20 hours with either soluble Aβ oligomers or Aβ fibers prepared from synthetic peptide. Cell viability was assessed using ATP content. Data are representative of three independent experiments and shown as mean +/- SEM. Aβ fibers were not toxic to the cortical neurons, validating that toxicity to these neuronal cultures is mediated by the oligomeric nature of the Aβ 1-42 peptide.

Fig. S7

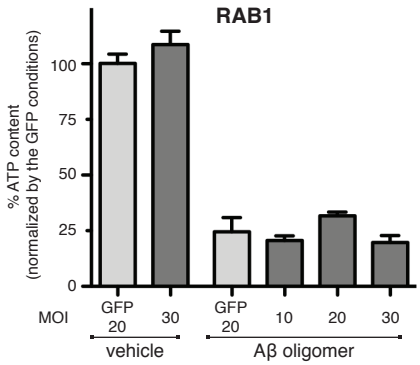

Fig. S7: RAB1 does not rescue Aβ induced toxicity in cortical neurons.
Cortical neuron cultures prepared from rat embryos at embryonic day 18 were cultured for 5 days, transduced, cultured for 13 days, and then incubated for 20 hours with 750nM of soluble Aβ oligomers prepared from synthetic peptide. Infection with a RAB1 lentiviral construct, a suppressor of neuronal α-syn toxicity, had no significant effect on Aβ oligomer toxicity. Cell viability was assessed by ATP content. Data are representative of three independent experiments and shown as mean +/- SEM (*: $p<0.05$; **: $p<0.01$, based on Dunnett's test).

Table S1. Propidium Iodide staining of strains expressing Aβ

We used propidium iodide (PI) staining to measure yeast cell death in our control strains and our strains expressing ssAβ 1-42. Of the 5000 cells counted for each sample, the % of cells dead (positive for PI) are indicated in the table above. These results show that there is no significant cell death caused by the expression of ssAβ 1-42 after 16 hours of growth in galactose.

	% of Total Cells Dead
Control: No PI	0.1
Control: Dead Cells	99.76
Control: p305 Vector	8.56
1 x ssAβ 1-42	21.08
ssAβ 1-42 s. strain 1	12.62
ssAβ 1-42 s. strain 2	18.72

Table S2. Suppressors and enhancers of Aβ toxicity identified in the yeast screen

We constructed yeast strains with an intermediate level of Aβ expression and corresponding toxicity that allowed us to identify in the same screen genes that alleviated or enhanced toxicity when overexpressed (see Materials and Methods). Aβ toxicity is likely to be influenced by mitochondrial function in neurons (*64, 65*). We took advantage of the fact that the extent to which yeast rely on mitochondrial respiration is carbon source dependent, thereby allowing us to test the full library of ORFs at different levels of respiration. In glucose, cells ferment and respiration remains low until all glucose is converted to ethanol. In galactose respiration is moderately active. In glycerol, cells are completely dependent on respiration for growth. To determine if the effect of putative modifiers on Aβ toxicity depended on the level of mitochondrial respiration, we conducted our screen on the three different carbon sources mentioned: glucose, galactose and glycerol. The expression of both ssAβ 1-42 and the library of yeast ORFs were under control of the *GAL1* promoter. To induce expression in glucose and glycerol, we employed a chimeric Gal4-ER-VP16 transcription factor that enables induction of the *GAL1* promoter through the addition of the estrogen estradiol (*49*). We plated the ssAβ strains carrying two plasmids, the Gal4-ER-VP16 transcription factor on one and individual yeast ORFs on the other, on media containing galactose, glucose + estradiol, or glycerol + estradiol. We also plated cells on glucose alone as a non-inducing growth control.

Suppressors increased growth relative to a vector control on the indicated conditions; enhancers decreased it (see Fig. S2B for an example of a screening plate). The function and localization of the gene products identified as modifiers are based on Saccharomyces Genome Database (SGD) gene summaries.

Only a few of the modifiers, specifically *SLA1, RTG3, NAB3, SLF1, FCY21, VPS9, GRR1, YBL086c, IVY1, PBS2, PKC1* and *MVP1,* were strongly affected by the state of mitochondrial respiration. However, the fact that most of the suppressors and enhancers were reproduced on all three media indicates the robustness of their affects on Aβ toxicity.